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Reproductive Effects of Low Acute Doses of Cadmium Chloride in Adult Male Rats

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Reproductive Effects of Low Acute Doses of Cadmium Chloride in Adult Male Rats

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Reproductive Effects of Low Acute Doses of Cadmium Chloride in Adult Male Rats. LASKEY, J. W., REHNBERG, G. L., LAWS, S. C., AND HEIN, J. F. (1984). *Toxicol. Appl. Pharmacol.* 73, 250–255. Adult male Sprague–Dawley rats were injected sc with cadmium (Cd, as cadmium chloride) in doses ranging from 1.6 to 152 μmol Cd/kg body weight (body wt). Fourteen days after dosing, animals were evaluated for reproductive damage. Evaluations for each animal included testes, seminal vesicles, and epididymides weights, vas deferens sperm concentration, and human chorionic gonadotropin (hCG) -stimulated serum testosterone concentration. Since 10 to 60% mortality occurred in the two highest dose groups (74 and 152 μmol/kg), no additional evaluations were conducted in these groups. The weights of the testes, seminal vesicles, and epididymides were reduced at least 40 to 50% in groups receiving 16 or 33 μmol Cd/kg while vas deferens sperm concentrations and hCG-stimulated serum testosterone concentrations were essentially zero. Significant depressions in the sperm concentrations and in the hCG-stimulated serum testosterone concentrations were found in animals receiving the two lowest doses (1.6 and 7.4 μmol Cd/kg) although no changes in tissue weights were observed in these animals. Curve–linear regression analyses for the dose responsiveness of these parameters demonstrated that serum testosterone concentration initially decreased at a rate of 19%/μmol Cd/kg, respectively, and was the most sensitive to Cd exposure. The initial rates of decrease for sperm concentrations and for seminal vesicles, testes, and epididymides weight were 6.45, 5.30, 4.19, and 2.45%/μmol Cd/kg, respectively, and were less responsive to Cd exposure than serum testosterone levels.

Effects of acute Cd exposure on the male reproductive system in the adult rat are well documented (Gunn *et al.*, 1961; Saksena *et al.*, 1977; Aoki and Hoffer 1978; Saksena and Lau 1979). The lower limit for testicular effects has been reported by Gunn *et al.* (1966) to be approximately 4 μmol (0.44 mg) Cd/kg. Most of the recent research uses dosing regimens of 1 mg Cd/kg or higher. Saksena *et al.* (1977), Lau *et al.* (1978), and Saksena and Lau (1979) have described the acute effects of large doses of Cd (9 to 45 μmol Cd/kg) on the reproductive and endocrinologic features in rats and hamsters 15 days postdosing. The fertility and endocrinologic effects described in those studies were accompanied by highly significant reductions in testicular mass of 50% or more. The parameters most affected appeared to be sperm concentration and the testosterone metabolic pathway. These effects were maximal 15 to 30 days after exposure.

The initial toxic effect on the testes of acute Cd exposure has been identified as vascular (Gunn *et al.*, 1963) and occurred within the first several hours following exposure. A dose of 30 μmol Cd/kg destroyed the veins of the pampiniform plexus. By 6 hr following 30 μmol Cd/kg body wt Timm and Schulz (1966) reported visible granules of cadmium in both the interstitial tissues and in the Leydig cells and by 18 to 24 hr there was extensive vascular

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damage. With an acute dose of 8 μmol Cd/kg body wt there was minimal hemorrhagic necrosis with most of the nonnecrotic damage to the seminiferous tubules rather than to the interstitial tissue (Meek, 1959; Mason *et al.*, 1964; Kar and Das 1960). Kar and Das (1960) also reported that even though there were no apparent degenerative changes in the interstitial tissue at this dose, accessory sex organs were reduced in size. Six days following a dose of 6 μmol Cd/kg body wt Gunn *et al.* (1966) observed some edema with no apparent histological differences in either the germinal or interstitial elements. The subtle effects of low acute Cd exposure on male endocrinologic and reproductive parameters have generally not been investigated. In this investigation adult male rats received a single, acute dose of Cd (as $CdCl_2$) ranging from 1.6 to 152 μmol Cd/kg, and reproductive evaluations were conducted 14 days after dosing. A preliminary evaluation of the hCG-stimulated serum testosterone response following acute exposure to 0.8 μmol Cd/kg body wt indicated that serum concentration was reduced to less than 1% of preexposure values within 24 hr and remained at this level through 15 days postexposure. Reproductive evaluations included testes, seminal vesicles, and epididymides weights, vas deferens sperm concentration, and human chorionic gonadotropin (hCG) -stimulated serum testosterone concentration. These reproductive parameters were statistically analyzed to determine their dose–response characteristics to acute Cd exposure.

METHODS

Eighty male Sprague–Dawley rats (Charles River Breeding Laboratory, Wilmington, Mass.), 70 days old, were randomly assigned to treatment groups, weighed, and injected sc with $CdCl_2$ dissolved in distilled water. Ten animals per group were injected with a single Cd dose of either 0, 1.6, 3.1, 7.4, 16, 33, 74, or 152 μmol/kg in 0.1 ml and were observed daily for toxic symptoms. Fourteen days after dosing, each animal was weighed and, 2 hr prior to death, injected sc with 100 IU of human chorionic gonadotrophin (hCG) (Sigma Chemical Co., St. Louis, Mo., stock No. CG 10, Lot No. 109C0158). Animals were killed with CO_2 gas, and 6 to 10 ml of blood was collected by cardiac puncture. Serum was prepared and frozen at −40°C until needed. Testes, epididymides, and seminal vesicles were removed, trimmed, and weighed. A sample of luminal fluid was expressed from the vas deferens, weighed, and immediately diluted in 10% phosphate buffered Formalin. Dilutions of this mixture were used for enumeration of spermatozoa with a Coulter Electronic Cell Counter (Model ZBI, Coulter Electronics, Inc., Hialeah, Fla.). The accuracy of the counting system was verified with a 4-C normal hematology reference control and monosized latex spheres (Coulter Electronics, Inc.) and by counting approximately 10% of the samples with a hemocytometer. Serum testosterone concentration was determined with a testosterone test set (Wein Laboratories, Inc., Succasunna, N.J., Catalog No. TS-333) and is essentially the method of Furuyama *et al.* (1979) as modified by Chen *et al.* (1971). Data were analyzed by a computer based statistical package (SAS) containing the general linear model (GLM) regression procedure (Helwig and Council, 1979) and Dunnett's (1955) test. Bartlett's (1937) test was also used to assess heterogeneity of variance.

RESULTS

Doses of 74 and 152 μmol Cd/kg resulted in mortalities of 10 and 60%, respectively, and no further evaluations were done on these groups. Groups of rats that received 33 μmol Cd/kg gained only 8 to 15 g while all other dose groups gained 30 to 60 g during the 14-day postexposure period (Table 1). Mean

TABLE 1

BODY WEIGHTS (g) AT DOSING AND 14 DAYS AFTER ACUTE Cd TREATMENT

Dose μmol Cd/kg (mg Cd/kg)	At dosing[a]	At death[a]	Percentage change
0 (0)	315 ± 10	369 ± 20	+17
1.6 (0.18)	341 ± 16	383 ± 28	+12
3.1 (0.34)	358 ± 22	391 ± 28	+9
7.4 (0.83)	369 ± 19	414 ± 32	+12
16 (1.80)	339 ± 15	366 ± 14	+8
33 (3.66)	333 ± 12	341 ± 14	+2

[a] Mean ± SD, $n = 10$ for each group.

TABLE 2

TISSUE WEIGHT EFFECTS 14 DAYS AFTER ACUTE Cd TREATMENT

Dose μmol Cd/kg (mg Cd/kg)	Testes (g)[a,b,c]	Seminal vesicles (g)[a,c]	Epididymides (g)[a,b,c]
0 (0)	3.03 ± 0.22 (0.82)	1.29 ± 0.20 (0.35)	0.95 ± 0.06 (0.26)
1.6 (0.18)	3.23 ± 0.24 (0.84)	1.22 ± 0.24 (0.32)	0.96 ± 0.08 (0.25)
3.1 (0.34)	2.89 ± 0.59 (0.74)	1.18 ± 0.23 (0.30)	0.93 ± 0.13 (0.24)
7.4 (0.83)	2.79 ± 0.85 (0.67)	1.42 ± 0.15 (0.34)	0.91 ± 0.13 (0.22)
16 (1.80)	1.61 ± 0.30[d] (0.44)	0.23 ± 0.10[d] (0.062)	0.49 ± 0.40[d] (0.13)
33 (3.66)	1.75 ± 0.14[d] (0.51)	0.18 ± 0.35[d] (0.053)	0.35 ± 0.05[d] (0.10)

[a] Mean ± SD, $n = 10$ for each group; number in parentheses is percentage of body weight.
[b] Heterogeneity of variance ($p < 0.01$).
[c] Significant treatment effect ($p < 0.001$).
[d] Mean significantly different from control group by Dunnett's test ($p < 0.05$).

weights of the testes, seminal vesicles, and epididymides were reduced 50 to 85% at doses of either 16 or 33 μmol Cd/kg (Table 2). Analysis of this organ weight data also indicated that significant ($p < 0.01$) heterogeneity of variance was present (Bartlett, 1937) in the intermediate dose groups (3.1 to 16 μmol Cd/kg).

In the 16 and 33 μmol Cd/kg groups, samples with intact sperm were not found and where evidence of sperm was found in these groups, only separated heads, tails, and other cellular debris were observed. Sperm concentration in the vas deferens (Table 3) was significantly depressed in the 7.4 μmol Cd/kg group. In the two lower exposure groups (1.6 and 3.1 μmol Cd/kg), there was approximately a 10 to 15% reduction which was not significantly ($p > 0.05$) different from the control value. Stimulated serum testosterone concentrations (Table 3) were significantly ($p < 0.05$) depressed in all Cd dose groups including the lowest dose group (1.6 μmol Cd/kg body wt). The Cd dose response descriptive statistics in Table 4 and Fig. 1 demonstrate that all parameters had significant ($p < 0.001$) dose–response characteristics when all doses (0 thru 33 μmol Cd/kg) are considered.

DISCUSSION

Results of the present study indicate that very low acute doses of Cd produce more sub-

TABLE 3

VAS DEFERENS SPERM AND SERUM TESTOSTERONE CONCENTRATIONS 14 DAYS AFTER CADMIUM TREATMENT

Dose μmol Cd/kg (mg Cd/kg)	Vas deferens sperm count ($\times 10^{-6}$/mg)[a,b]	hCG-stimulated serum testosterone[a,b,c] (ng/ml)
0 (0)	1.79 ± 0.27	19.59 ± 5.45
1.6 (0.18)	1.63 ± 0.18	16.47 ± 1.96[d]
3.1 (0.34)	1.54 ± 0.15	9.80 ± 3.87[e]
7.4 (0.83)	0.94 ± 0.36[e]	13.08 ± 2.43[e]
16 (1.80)	0[e]	0.43 ± 0.18[e]
33 (3.66)	0[e]	0.31 ± 0.11[e]

[a] Mean ± SD, $n = 10$ for each group.
[b] Significant treatment effect ($p < 0.001$).
[c] Heterogeneity of variance ($p < 0.01$).
[d] Mean is significantly different from control group by Dunnett's test ($p < 0.05$).
[e] Mean is significantly different from control group by Dunnett's test ($p < 0.01$).

TABLE 4

CURVE–LINEAR FIT STATISTICS[a]

Parameter	Statistic	Estimate	Standard error of estimate	*R* Square
		0 to 33 μmol Cd/kg—analyzed as % of control		
Testes weight	Intercept	108.2	3.5	0.61
	C1	−4.19[b]	0.71	
	C2	0.079[b]	0.021	
Seminal vesicles weight	Intercept	108.6	4.9	0.71
	C1	−5.3[b]	0.99	
	C2	0.071[c]	0.029	
Epididymides weight	Intercept	102.7	3.56	0.60
	C1	−3.45[b]	0.89	
Stimulated serum testosterone	Intercept	100.4	6.15	0.73
	C1	−19.0[b]	4.76	
	C2	2.65[b]	0.82	
	C3	−0.11[b]	0.035	
		0 to 7.4 μmol Cd/kg—analyzed as % of control		
Stimulated serum testosterone	Intercept	103	5.75	0.49
	C1	−22.3[b]	4.19	
	C2	2.28[b]	0.51	
Vas deferens sperm concentration	Intercept	102	2.57	0.63
	C1	−6.45[b]	0.82	

[a] Parameter = Intercept + (C1•Dose) + (C2•Dose^2) + (C3•Dose^3), where C1 is the linear effect coefficient and C2 and C3 are the curvature effect coefficients. *R* square is equal to the regression sums of squares divided by the total sums of squares and indicates that the variation in the parameter is reduced by (*R* Square•100) percent when model relation to dose is utilized. (Neter and Wasserman, 1974).

[b] $p < 0.01$.

[c] $p < 0.02$.

tle alterations in testosterone and sperm production than were previously reported. This study demonstrates that assessments of male reproductive damage, especially minimal damage, may require multifaceted evaluations of all areas of potential damage. In this study a careful examination of selected morphologic and endocrinologic parameters previously identified by other investigators as responsive to relatively large single doses of Cd (9 μmol Cd/kg or greater), was conducted over a wide range (1.6 to 33 μmol Cd/kg) of Cd exposures. The linear effect coefficient of the dose–response curve (C1, Table 4) is an indicator of the magnitude of a parameter's initial sensitivity to acute Cd exposure. When all doses (0 thru 33 μmol Cd/kg) are considered, the parameters would be ordered as: stimulated serum testosterone > seminal vesicles weight > testes weight > epididymides weight. When only the low Cd doses (0 to 7.4 μmol Cd/kg) are considered, the order would be: stimulated serum testosterone > sperm concentration, with the organ weights being not significantly ($p > 0.10$) responsive.

Our results are in agreement with those of Saksena *et al.* (1977) in that at doses of greater than 9 μmol Cd/kg, vas deferens sperm concentrations were reduced to essentially zero by 15 days postexposure while endogenous

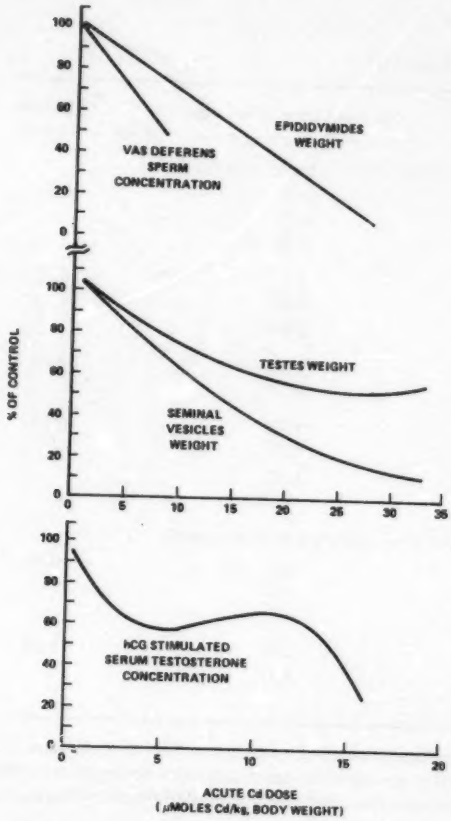

FIG. 1. Plot of the fitted regression curves with the fit statistics in Table 4.

serum testosterone concentration was reduced to 60% of the control value (from 1.46 to 0.85 ng/ml). In another study Saksena and Lau (1979) indicated that this same dosing regimen resulted in a 50% reduction in endogenous serum testosterone 35 days postexposure while *in vitro* testosterone release was depressed to 11 and 41% of control values at 10 and 30 days postexposure, respectively. In addition, their study also showed that the *in vitro* hCG stimulation of testicular tissue 14 days post-acute Cd exposure resulted in a 25% reduction in testosterone release when compared to control values.

This study describes the dose–response characteristics in male rats following exposure to low acute doses of Cd. Examination of the individual linear effect coefficient, which is the initial response to the acute Cd exposure, identified simulated serum testosterone concentration as the most responsive reproductive parameter, initially decreasing from the control value at a rate of 19 to 22%/μmol Cd/kg. This reduction in the stimulated serum testosterone concentration in conjunction with the studies of Saksena and Lau (1979) indicates that the reduction is due to testicular and not CNS toxicity. Sperm concentration, seminal vesicles weight, testes weight, and epididymides weight were less responsive and decreased at a rate of 6.45, 5.30, 4.19, and 3.45%/μmol Cd/kg, respectively.

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